

RESEARCH ARTICLE

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Novel catalytic fluorescence method for speciative determination of chromium in environmental samples

Sunil Adurty* and Jagadeeswara Rao Sabbu

Abstract

Background: Thiourea derivatives act as promising chemosensors for sensing transition metal ions. 1-(2-hydroxyphenyl) thiourea (HPTU) is one such chromophore that has potential for metal ion sensing. The current investigation reports the sensing of chromium species using transition metal-oxo-based reaction of 1,2-hydroxyphenylthiourea.

Methods: The catalytic effect of chromium (III) and chromium (VI) on the oxidation of HPTU was studied. The reaction was followed spectrofluorimetrically by measuring the fluorescence intensities of the reaction product at $\lambda_{\text{ex}} = 416$ and $\lambda_{\text{em}} = 520$ nm, respectively.

Results: Under the optimum analytical conditions, HPTU acts as a chromogenic sensor for the detection of chromium species in nano-gram levels with a determination range of 0.3 to 250 ng/mL.

Conclusions: The methods are fairly sensitive, and the role of activators and sensitizers in enhancing the catalysis was studied. Interference due to various cations and anions in the experiment was investigated. The proposed method was applied to environmental samples for the analysis of chromium content.

Keywords: 1-(2-hydroxyphenyl) thiourea; Chromium; Speciative determination; Catalytic fluorescence

Background

The toxicological and biological characteristics of many transition metals like chromium are related to their chemical forms. A great interest in chromium speciation originates from applications of this metal in various industrial activities such as tanning of leather, electroplating, pigment production and wood preservation. Owing to these industrial processes, large amounts of chromium compounds discharge into the environment, which can affect biology and ecology of the environment. Therefore, speciation analysis of chromium is of great importance to assess pollution levels. Chromium mainly exist in two oxidation states, i.e. Cr(III) and Cr(VI). Cr(III) appears to be one of the essential elements for the proper functioning of living organisms, effective in the maintenance of normal glucose, cholesterol and fatty acid metabolism, while water soluble Cr(VI) is toxic to human and living organisms and

was found to be carcinogenic. Due to the different toxicities of Cr(III) and Cr(VI), and due to their association in many sample matrices, it is necessary to develop methods where both species can be determined simultaneously (Kotas and Stasicka 2000).

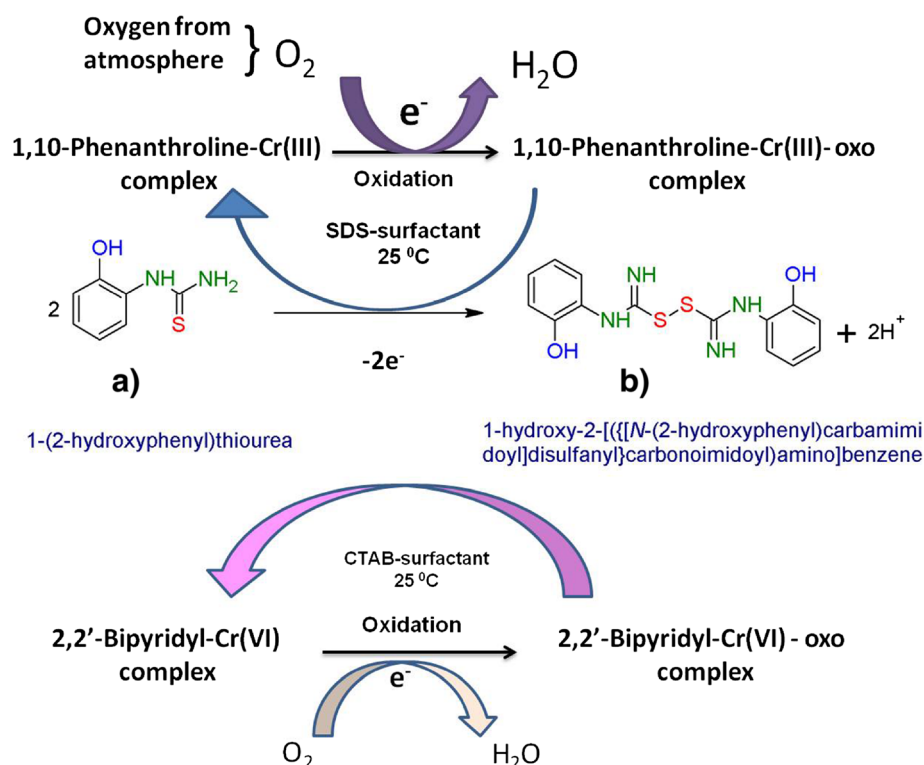
In the past years, various analytical techniques such as atomic absorption spectrometry (AAS) (Karosi et al. 2006; Ren et al. 2007) spectrophotometry (Wu et al. 2007), stripping voltammetry (SV) (Grabarczyk et al. 2006), inductively coupled plasma-mass spectrometry (ICP-MS) (Sun et al. 2006), inductively coupled plasma-optical emission spectrometry (ICPOES) (Schramel et al. 1992), and high performance liquid chromatography (HPLC) (Padaruskas and Naujalis 1998) have been successfully used to determine chromium in various samples. An extensive coverage of the available methods for chromium determination was put forth by Gomez and Callao, including the various types of sample matrices selected for the determination (Gomez and Callao 2006). Reagents such as bis-[2-hydroxy-1-naphthaldehyde] thiourea (Kiran et al. 2008), quercetin (Hosseini and Belador, 2009), chromotropic

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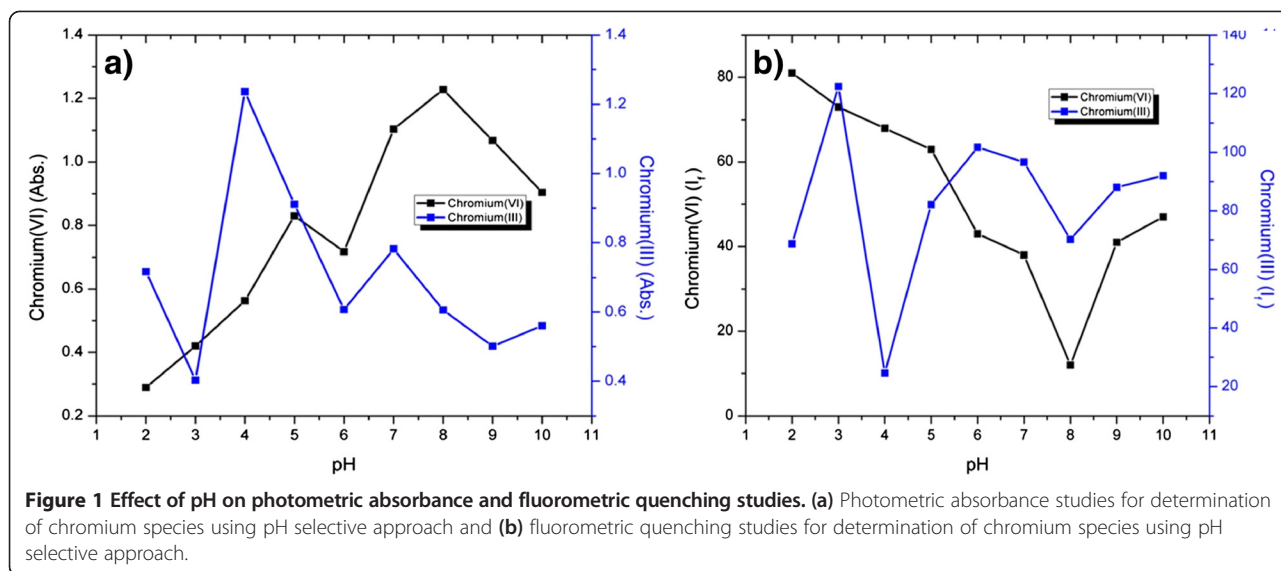
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acid (CA) (Themelis et al. 2006), bis (salicylaldehyde) orthophenylenediamine (BSOPD) (Arancibia et al. 2012 and Soomro et al. 2011), etc. have been used. Methods such as solidified floating organic drop microextraction (SFODME) in combination with graphite furnace atomic absorption spectrometry (GFAAS) (Moghadam et al. 2011), cloud point extraction (CPE) using diethyldithiocarbamate (DDTC) as the chelating agent (Yildiz et al. 2011), solid phase extraction procedure using ICP-MS (Guerrero et al. 2012), fluorescence method using tetraphenylphosphonium bromide (TPP^+Br^-) (El-Shahawi et al. 2011), EPA methods 3060A and 3052 (Martone et al. 2013), a disposable dual screen-printed electrode method using batch and flow analysis (Sánchez-Moreno et al. 2010), p-aminoacetophenone and phloroglucinol (Parmar et al. 2010), ultrasound-assisted cloud point extraction (UACPE) (Hashemi and Daryanavard 2012), electrospray ionization mass spectrometry using CYDTA (Hotta et al. 2012), HPLC and preconcentration by CPE with 1-(2-thiazolyazo)-2-naphthol (TAN) as the chelating agent (Wang et al. 2010), ytterbium (III) hydroxide (Duran et al. 2009), mixed-micelle cloud point extraction using electrothermal atomic absorption spectrometry (ET-AAS) (Ezoddin et al. 2010), room temperature ionic liquids (RTILs) for hollow fiber liquid phase microextraction (HF-LPME) combined with flame atomic absorption spectrometry (FAAS), etc., have been developed (Zeng et al. 2012).

It is evident from the literature that hexavalent chromium compounds are 10 to 100 times more toxic than trivalent chromium compounds when administered orally. The World Health Organization (WHO) and the European Community Directive (ECD) for drinking water has set the limit of total chromium not exceeding $50 \mu\text{g L}^{-1}$, while the maximum concentration criterion for Cr(VI) in freshwater is $16 \mu\text{g L}^{-1}$, sea water contains between 0.1 and $0.5 \mu\text{g L}^{-1}$ and unpolluted river water from 0.3 to $0.6 \mu\text{g L}^{-1}$. Since the concentration of chromium, mainly Cr(VI), is very low in many natural waters, a highly sensitive method is required for its speciation (Arancibia et al. 2012). Innumerable techniques and methods were reported for chromium speciation using highly sophisticated equipment. Among the plethora of the methods, photometric and fluorometric methods are comparatively simple and relatively sensitive. Hence, speciative determination of chromium by parameter selective approach using a novel thiourea derivative was proposed. This paper is an extension of the work carried out and formerly reported by the authors (Sunil and Rao 2015). Many analytical methods reported for chromium determination are based on the Beer's law and the metal ligand complexation studies by addition of oxidizing agents like hydrogen peroxide. But, the reaction utilized in the current study is a unique catalytic reaction in itself because the reagent synthesis is very



Scheme 1 The proposed mechanism for the process of catalytic effect of chromium species on HPTU.

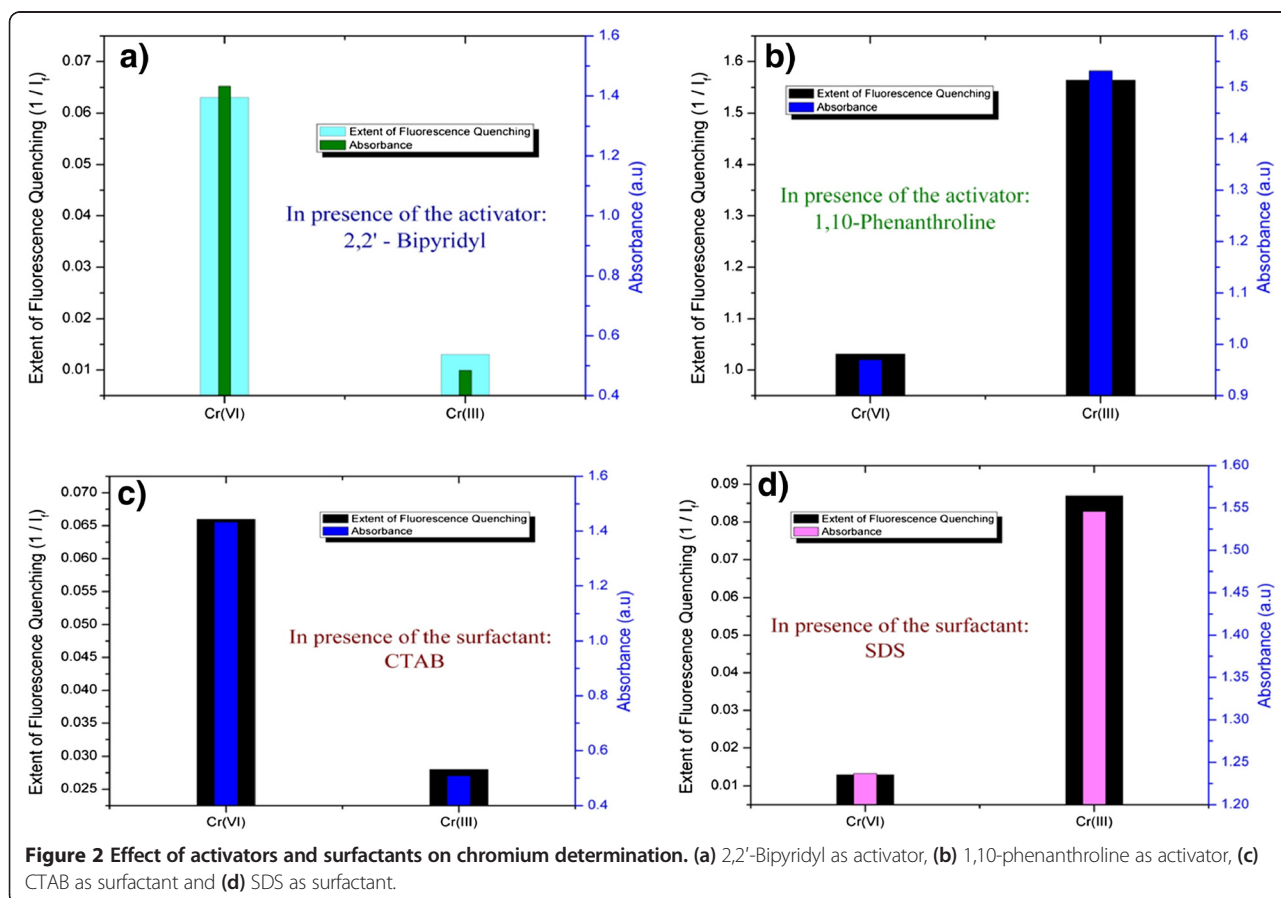


simple and there is no need to add any oxidizing agent. There are no pre-concentration methods involved, the entire study can be done at normal room temperature, no inert atmosphere required, it is bench-stable and the methods are cost-effective.

Results and discussion

Proposed mechanism

The proposed mechanism describes that chromium species complexes with activators like 1,10-phenanthroline, 2,2'-bipyridyl resulting in the formation of organo-metallic



complexes. These complexes reacts with atmospheric oxygen to form highly reactive oxo-complexes, which are also unstable and short lived. This reactive oxo species attains high oxidation states which are unstable and in order to get back to their stable states from the highly unstable ones, they would accept the electrons from the HPTU. This process enables the conversion of HPTU to its disulphide. In this way, the catalytic action of chromium species in the presence of activators and surfactants was proposed (Scheme 1).

Experimental observations

The speciative determination of chromium(III) and chromium(VI) was done using photometric and fluorometric

methods. In both developed methods, three parameters were proposed, which can selectively determine each species. The three parameters are the following: pH, activator and surfactant. HPTU was used as a chromogenic reagent to selectively sense a specific species at a defined pH. With respect to the observations pertaining to pH study, it was noticed that HPTU reaction was catalysed by chromium(III) ions only in the pH range of 3.5 to 4.2. Similarly, chromium(VI) ions catalyse HPTU reaction only in the pH range of 7.8 to 8.4. Thus, by selectively maintaining the respective pH ranges, one can easily and selectively determine the chromium species (Figure 1). The next parameter that was utilized in the determination was the usage of activators like pyridine, 2-aminopyridine, 2,2'-bipyridyl,

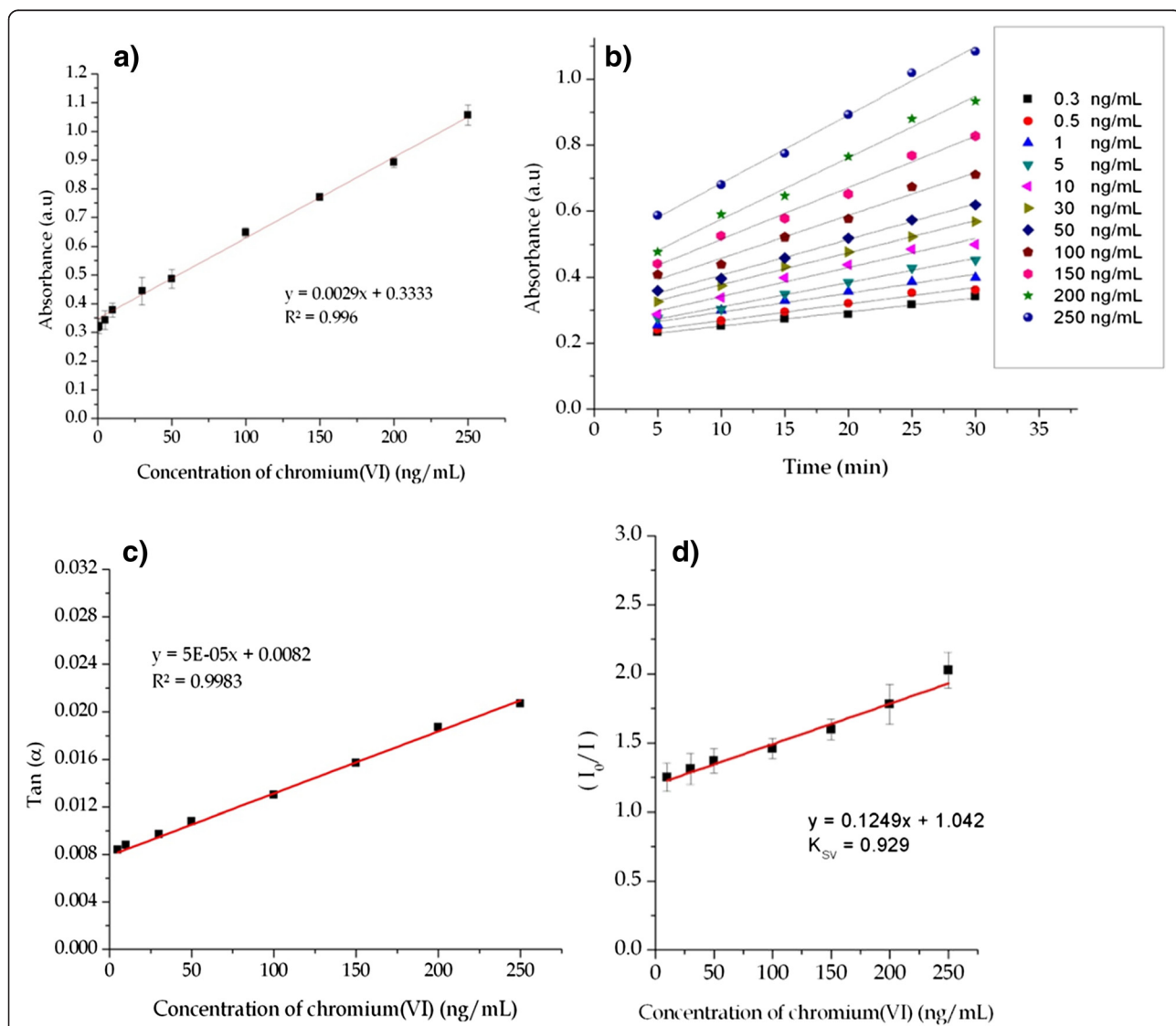
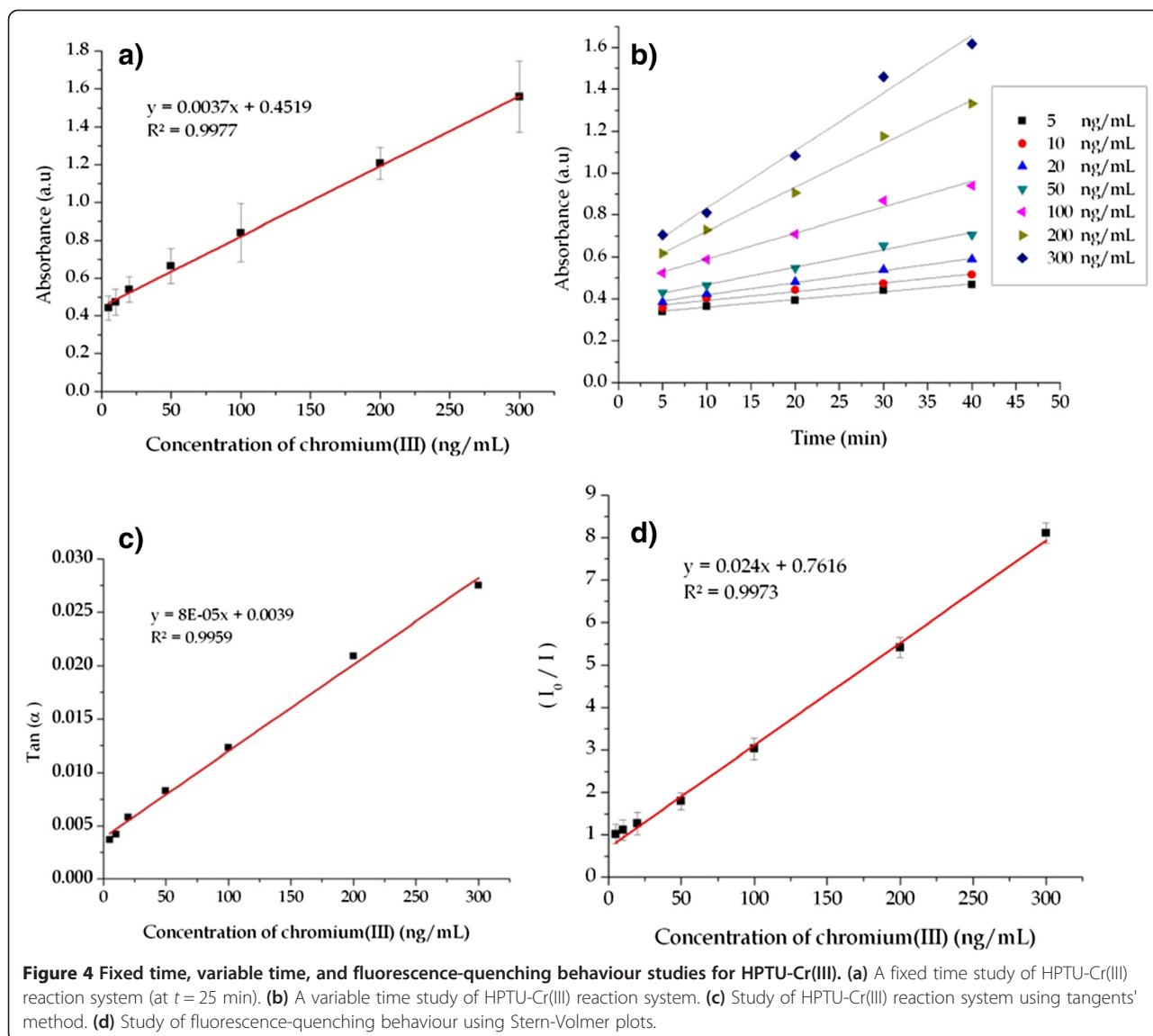


Figure 3 Fixed time, variable time, and fluorescence-quenching behaviour studies for OHPTU-Cr(VI). (a) A fixed time study of OHPTU-Cr(VI) reaction system (at $t = 25$ min). (b) A variable time study of OHPTU-Cr(VI) reaction system. (c) Study of HPTU-Cr(VI) reaction system using tangents' method. (d) Study of fluorescence-quenching behaviour using Stern-Volmer plots.



1,10-phenanthroline, etc. In the case of chromium(III), 1,10-phenanthroline acts as a very good activator, by selectively activating chromium(III) in the acidic pH range. Similarly, 2,2'-bipyridyl selectively activates chromium(VI) in the basic pH range. Thus, by the selective activation of HPTU reaction using Cr(III) - 1,10-phenanthroline and Cr(VI) - 2,2'-bipyridyl in acidic and basic pH ranges, the chromium species can be determined (Figure 2a,b).

The third parameter that was followed is the selective use of surfactants in addition to activators. In this study, it was observed that the anionic surfactant, sodium dodecyl sulfate (SDS) encapsules the Cr(III) - 1,10-phenanthroline moiety and acts as a micro reaction centre facilitating the oxidation of HPTU. Similar in the other case, the cationic surfactant, CTAB encapsules the Cr(VI) - 2,2'-bipyridyl moiety in basic pH forming a micro reaction centre and

Table 1 Determination of chromium (III) species using the proposed and standard AAS methods

Sample	Amount of Cr (III) added (ng)	Experimental values for Cr (III) (ng) ($n = 3$)		
		Photometric	Fluorometric	AAS
Tap water	150.0	149.7 ± 0.04	149.4 ± 0.06	149.6 ± 0.06
Rain water	130.0	132.8 ± 0.08	132.5 ± 0.07	132.3 ± 0.05
Drainage water	100.0	108.6 ± 0.04	108.9 ± 0.05	108.9 ± 0.07

Table 2 Determination of chromium (VI) species using proposed and standard AAS methods

Sample	Amount of Cr (VI) added (ng)	Experimental values for Cr (VI) (ng) (n = 3)		
		Photometric	Fluorometric	AAS
Tap water	180.0	179.4 ± 0.05	179.6 ± 0.08	179.5 ± 0.07
Rain water	140.0	166.8 ± 0.08	166.5 ± 0.04	163.7 ± 0.05
Drainage water	120.0	138.4 ± 0.04	138.8 ± 0.05	138.5 ± 0.07

facilitates the catalytic reaction (Figure 2c,d). Using the above parameters, the chromium species were determined selectively in the range of 0.3 to 250 ng/mL. Linear plots were obtained in the absorbance study by following fixed time and tangents' methods. Similarly, fluorescence studies also inferred the quantitative linear relationship through Stern-Volmer plots (Figures 3d and 4d).

Interference by foreign ions

In the speciative determination of chromium, the effect of associated metal ions and anions was studied. It was observed that metal ions like Na(I), Ba(II), Bi(III), Sb(III), V(V), W(VI), Ru(III) and Re(II) showed no interference even up to 1,500-folds. Other metal ions like Mn(II), Co(II), As(V), Pb(II), Hg(II), Cd(II), Zn(II), Pd(II), Ni(II), Zn(II), Ca(II), Mg(II), Zr(IV), Rh(III), Os(IV), Au(III), Mo(IV), Al(III), Ir(III), Pt(II) and Ag(I) do not interfere up to 800-folds at the pH of 8.0. Fe(II) and Cu(II) interfere up to 20-folds, which can be masked by the addition of tartarate and thiosulphate, respectively.

Analytical application of the method

The developed methods were applied to water samples like drainage water, tap water and rain water. It was found that the concentration of chromium(VI) was comparatively more in the case of rain water and drainage water. This can be attributed to the leaching of chromium from paints, pipelines and other sources of pollution. The proposed photometric and fluorometric methods are in agreement with the experimental data obtained from standard atomic absorption spectrometry (AAS) method (Tables 1 and 2).

The obtained results of water sample analysis using absorbance and emission studies have been statistically treated using ANOVA (Tables 3 and 4). A high degree of linear relationship between both methods is very much evident from the Pearson correlation values closer

to 0.999. The F_{Ratio} value calculated from ANOVA: single factor is lesser than 1 and F_{Ratio} is lesser than F_{Crit} in both the experiments, inferring no significant difference between the three pertained methods. Also, the analysis shows that the type of sample chosen has no significant effect on the developed methods.

Experimental

Chemicals and instrumentation

Analytical grade chemicals with high purity purchased from Sigma-Aldrich Chemicals Ltd., Bengaluru, India, were used in the investigation. Similarly, HPLC-graded solvents purchased from Merck, Mumbai, Maharashtra, India, were used appropriately. The standard prescribed procedure was employed for the preparation of buffer solutions (Vogel 1961). The pH adjustments were appropriately done using Micropro pH meter (Techno Instruments Co., Bangalore, India). The instruments such as PerkinElmer LS-55 fluorimeter (PerkinElmer, Waltham, MA, USA) and Hitachi-2001 spectrophotometer (Hitachi Ltd, Chiyoda-ku, Japan) were utilized for experimental studies. Mettler Toledo AB204-S (Mettler-Toledo, LLC, Columbus, OH, USA) was utilized for weighing purposes.

Reagents

In an 'A' grade 100-mL volumetric flask, 100.0361 mg of chromium (III) chloride was dissolved thoroughly and the resultant solution was standardised with EDTA. A stock solution of 6.3 mM (1.0 mg/mL) of chromium (III) was obtained.

In a 100-mL volumetric flask, 100.0704 mg of potassium dichromate was weighed, and deionised water was added up to the mark. The prepared solution was standardised by titrating with Mohr's salt, using diphenylamine as an indicator along with 2.0 mL of 1:1 H_3PO_4 . A stock solution of 3.4 mM (1.0 mg/mL) of chromium (VI) was obtained.

Table 3 Statistical analysis of the obtained data for chromium (III) species

Statistical parameter	Calculated value
F_{Crit}	5.14
F_{Ratio}	2.39E-05
p -value	0.999

Table 4 Statistical analysis of the obtained data for chromium (VI) species

Statistical parameter	Calculated value
F_{Crit}	5.14
F_{Ratio}	0.0023
p -value	0.997

1.0407 g of 1,10-phenanthroline was weighed and dissolved in ethanol in a 100-mL volumetric flask to get 0.58 mM of 1,10-phenanthroline reagent.

By weighing 1.0207 g of 2,2'-bipyridyl in a 100-mL volumetric flask, 0.66 mM of 2,2'-bipyridyl reagent was prepared, and ethanol was added up to the mark.

In distilled water using a 100-mL volumetric flask to get a 1% SDS solution, 1.0 g of SDS was weighed and dissolved. In a 100-mL volumetric flask, 1.0 g of CTAB was weighed and dissolved in distilled water to get 1% CTAB solution.

The above recommended experimental procedures were followed as described (Vogel 1961).

Methods

The reagent, HPTU, was synthesized (Sunil and Rao 2012a) and the analytical parameters, and their influence was studied systematically. A solution of 1.0 mL of acetate buffer of pH = 4.0 and appropriate amount (5 to 300 ng/mL) of chromium (III) solution was pipetted in to a 10.0-mL volumetric flask, followed by 1.0 mL of 1,10-phenanthroline and 1.0 mL of 1% SDS. The temperature was maintained at 25°C. Two milliliters of HPTU (1 mg/mL) solution was then added, and millipore water was added up to the mark. The resulting reaction mixture was transferred into 10-mm quartz cuvettes. The photometric measurements were recorded at $\lambda_{\text{Max}} = 416$ nm. The fluorescence emission measurements were recorded at $\lambda_{\text{Em}} = 520$ nm upon excitation at $\lambda_{\text{Ex}} = 416$ nm, respectively, at 30 min. The blank experiments were repeated by following the same procedure to obtain relative fluorescence intensity I_0 and the value of I_0/I was calculated. The calibration graph was plotted and the method was applied for the determination of chromium in waste water and plant samples. Water samples were boiled, treated with concentrated nitric acid and then filtered to remove organic particulate matter before using for the analysis. The digestion for plant sample was done as mentioned (Sunil and Rao 2012b). The same procedure was followed by taking chromium (VI) solution. Here, 2,2'-bipyridyl was used as an activator and 1% CTAB as a surfactant.

Conclusions

The results suggest that the speciative determination of chromium species was achieved by the selective application of parameters of pH, activators and surfactants using photometric and fluorometric techniques. Chromium(III) was determined in the pH range of 3.5 to 4.2 and in presence of 1,10-phenanthroline as an activator and SDS as a surfactant. Chromium(VI) was determined in the pH range of 7.8 to 8.4 and in presence of 2,2'-bipyridyl as an activator and CTAB as a surfactant. The methods are fairly sensitive with a determination range of 0.3 to

250 ng/mL. The proposed methods were applied to tap water, rain water and drainage water with satisfactory results.

Competing interests

The authors declare no competing interests.

Authors' contributions

Sunil has performed the entire experimental, analytical work and prepared the draft of the manuscript. The supervision of this work was done by Rao. All authors read and approved the final manuscript.

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